1 up

NASA TECHNICAL TRANSLATION NASA TT F-15,359

QUANTITATIVE DETERMINATION OF FIBRINOLYSIN IN STAPHYLOCOCCI WITH A FIBRINOGEN COAGULASE SOLUTION

G. Hentschel and H. Blobel

Translation of "Quantitative Fibrinolysinbestimmung bei Staphylokokken mit einer Fibrinogen-Koagulaselösung," Zentralblatt für Bakteriologie Parasitenkunde. Infekt. und Hygiene. Erste Abteilung: Originale (Stuttgart), Vol. 204, No. 3, pp. 322-324, 1967

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 FEBRUARY 1974

STANDARD TITLE PAGE

1. Report No. NASA TT F-15,359	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle QUANTITATIVE DETERMINATION OF FIBRINOLYSIN IN STAPHYLOCOCCI WITH A FIBRINOGEN COAGULASE SOLUTION		5. Report Date February 1974 6. Performing Organization Code
7. Author(s) G. Hentschel and H. Blobel, Institute for Bacteriology and Immunology of the Julius Liebig University, Giessen		8. Performing Organization Report No. 10. Work Unit No.
9. Performing Organization Name and Address Leo Kanner Associates Redwood City, California		11. Contract or Grant No. NASw-2481 13. Type of Report and Period Covered Translation
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration, Washington, D.C. 20546		14. Sponsoring Agency Code

15. Supplementary Notes

Translation of "Quantitative Fibrinolysinbestimmung bei Staphylokokken mit einer Fibrinogen-Koagulaselösung," Zentralblatt für Bakteriologie Parasitenkunde. Infekt. und Hygiene. Erste Abteilung: Original (Stuttgart), Vol. 204, No. 3, pp. 322-324, 1967

16. Abstract

A tube test for the quantitative determination of fibrinolysin was developed. The substrate was a 3% fibrinogen solution, which had been clotted by staphylococcal coagulase. The reactions were recorded after 3 hours at 37°C.

17. Key Words (Selected by Author(s))	18. Distribution Statement
	Unclassified-Unlimited

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	46	3,00

NASA-HQ

QUANTITATIVE DETERMINATION OF FIBRINOLYSIN IN STAPHYLOCOCCI WITH A FIBRINOGEN COAGULASE SOLUTION

Gerd Hentschel and Hans Blobel,
Institute for Bacteriology and Immunology of the Justus Liebig University, Giessen,

The tube test described by Tillet and Garner [3] was initially used by several authors [1, 2] for quantitative determination of fibrinolysin in staphylococci. The corresponding substrate was human or rabbit plasma precipitated by $CaCl_2$ or thrombin. The fibrinolysin solution to be tested was diluted to half the concentration. Later, instead of plasma, Kline [4] used purified fibrinogen which had been precipitated by thrombin. This provided largely constant experimental conditions. /322*

Furthermore, purified fibrinogen does not contain any antibodies which could inhibit fibrinolysis. However, the presence of antibodies must be anticipated in plasma. The fibrinolysin activity was estimated by the rate of fibrin solvation. However, this requires that the reactions be monitored constantly. This drawback has been eliminated in the procedure to be described. A further, obvious idea is to use the conversion of fibrinogen to fibrin by staphylococcus coagulase for the investigation of fibrinolysin in connection with the virulence-factor analysis in staphylococci.

Material and Methods

A fibrinogen-containing solution was employed as an indicator for the fibrinolysin determination. Purified fibrinogen (bovine fraction 1, Armour Pharmaceutical Company, Kankakee, Ill.)

[1] This research was conducted with the support of the German Research Society.

* Numbers in the margin indicate pagination in the foreign text

was dissolved in 0.14 M NaCl in a concentration of 3%. Rabbit plasma was added as a cofactor in a final concentration of 2% to the fibrinogen solution. The fibrinogen-plasma solution was sterilized by Seitz filtration. Coagulase was extracted from a staphylococcus-broth culture by the method of Blobel et al. [5] and quantitatively determined. One coagulase unit (C.U.) is the amount of coagulase which just coagulated 0.4 ml of fibrinogen-plasma solution after 3-hour incubation at 37°C. The purified coagulase was dissolved in 0.14 M NaCl, adjusted to 1 C.U. per milliliter, and sterilized by Seitz filtration.

The fibrinolysin solution to be tested was diluted twofold with 0.14 M $CaCl_2$. To each 0.25 ml of these diluted solutions were added 0.4 ml of the fibrinogen solution and 0.1 ml of the coagulase solution. This was carried out in direct succession in agglutination tubes. All investigations were carried out twice.

Results /323

The test series were investigated after various periods of incubation. The most favorable reading point was found to be following 3-hour incubation at 37°C. After this period, the tubes in which the fibrinolysin was still active had liquid contents. The other tubes showed solid coagula (see Fig. 1).

Fibrinolysin activity was determined in a twofold dilution sequence in fibrinolysin units (F.U.). One F.U. was the smallest quantity of fibrinolysin with which the fibrinogen-coagulase solution remained completely liquid after a 3-hour incubation at 37°C. If this endpoint were e.g. reached at a fibrinolysin dilution of 1:100, the initial solution would thus have contained 100 F.U. per 0.25 ml.

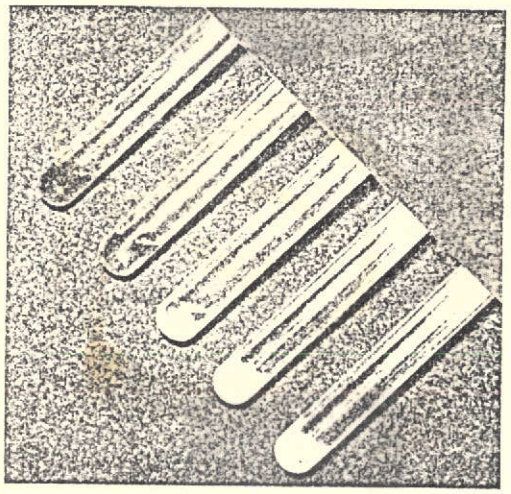

Fig. 1. From left to right: Tubes 1, 2, and 3 -- fibrinolysin; tubes 4 and 5 -- coagulase.

Discussion

The quantiative determination of fibrinolysin is an important prerequisite for an intensive investigation of this enzyme, which may be significant in the pathogenetic mechanism in staphylococci. A fibrinogen-coagulase solution was selected as a substrate for the fibrinolysin determination. Using the fibrinogen solution instead of plasma particularly eliminated the possibility of reaction inhibition due to antibodies. The conversion of fibrinogen to fibrin was brought about by coagulase, in order to largely simulate the probable events in the infection process. The endpoints of the reactions with the substrate described could be clearly recognized. These methods might also be used for measuring antibodies against fibrinolysin.

REFERENCES

1. Neter, E., "Fibrinolytic, anticoagulating and plasma-clotting properties of staphylococci," J. Bact. 34, 243-254 (1937).

2. Pillet, J., Mercier, G. and Pery, R., "Investigations on staphylococcus fibrinolysis," Ann. Inst. Pasteur 75, 458-471 (1948).

3. Tillet, W. S. and Garner, P. D., "The fibrinolytic activity of hemolytic streptococci," J. Exp. Med. 58, 485-502 (1933).

4. Kline, L. D., "Enzymes in blood clotting," in: Colowick-Kaplan, Method in Enzymology 2, 139-166 (1955).

5. Blobel, H., Berman, D., and Simon, J., "Purification of staphylocoagulase," J. Bact. 79, 807-815 (1959).